



UNIVERSITI PUTRA MALAYSIA

**PRODUCTION OF LACTIC ACID AND
POLYHYDROXYALKANOATES (PHA)
FROM RESTAURANT WASTE**

HAFIZAH KASSIM

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**PRODUCTION OF LACTIC ACID AND POLYHYDROXYALKANOATES
(PHA) FROM RESTAURANT WASTE**

By

HAFIZAH KASSIM

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfillment of the Requirements for Degree of Doctor of Philosophy**

December 2001



DEDICATION

Husband; Mohd Noor Ramlan:

Thanks for your love, patience, understanding and support

Children; Ahmad Wahidi, Ahmad Aizad and Afiqah Auni:

Sorry for neglecting you and thank you for being so understanding. You are the inspiration of my life.

Mak, Abah and family:

Thank you for the support and help

Abstract of a thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

**PRODUCTION OF LACTIC ACID AND POLYHYDROXYALKANOATES
(PHA) FROM RESTAURANT WASTE**

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December 2001

Chairman: Associate Professor Mohd. Ali Hassan, Ph.D.

Faculty: Food Science and Biotechnology

In this study, restaurant waste was used as a substrate for the production of lactic acid and polyhydroxyalkanoate (PHA) by *Ralstonia eutropha* (formerly known as *Alcaligenes eutrophus*). PHA production was achieved via a two-stage process; lactic acid production from restaurant waste followed by PHA production from lactic acid. In the first stage, the study was focused on optimization of culture condition for lactic acid production in batch anaerobic treatment. These treatments were carried out in a 2L stirred-tank bioreactor with 1L working volume. The pH was controlled using a pH controller by adding 3M NaOH automatically. The lactic acid produced was determined by HPLC method. Optimization for best conditions of lactic acid production were done on temperature, duration at controlled pH 7, seeding with appropriate inoculum and culture under sterile and non-sterile conditions. Lactic acid was dominantly produced (70-99%) during most of the anaerobic treatments followed by acetic (1-20%), butyric (0-8%) and propionic acid (0-1%). Among the three different temperatures investigated i.e. 30°C, 37°C and 45°C, 37°C was the best for lactic acid production (17 g/L). Studies on

the effect of duration at controlled pH 7 showed that by controlling pH 7 for 12 hours, higher lactic acid was produced (40 g/L). Production of lactic acid was reduced when pH 7 was controlled for longer period due to methanogenesis. Seeding with *Lactobacillus rhamnosus* results in higher production of lactic acid (67 g/L). Increased concentration of lactic acid was detected under sterile condition. In this study, the maximum concentration of lactic acid produced from restaurant waste was 97.7 g/L when the temperature was controlled at 37°C, controlled pH 7 throughout the experiment and seeded with *L. rhamnosus* under sterile condition.

The supernatant collected from the treatment of highest lactic acid production was used in the second stage for the production of polyhydroxyalkanoate. PHA was produced by *Ralstonia eutropha* strain ATCC 17699 in a 2L bioreactor (1L working volume) in batch culture, using lactic acid as the sole carbon source during the production phase. The bacteria was first precultivated in a nutrient-rich medium for 24 h before inoculation into the production medium i.e. treated restaurant waste containing 20-25 g/L lactic acid as the carbon source. Initial inoculum cell density was kept high at 4-5 g/L. The maximum PHA concentration obtained after 70h was about 10 g/L, corresponding to 97% (g/g) of cell dry weight.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains.

**PENGHASILAN ASID LAKTIK DAN POLYHYDROXYALKANOATE
DARIPADA SISA BUANGAN RESTORAN**

Oleh

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Pengerusi: Profesor Madya Mohd. Ali Hassan, Ph.D

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Di dalam kajian ini, sisa buangan restoran digunakan sebagai substrat bagi penghasilan asid laktik dan polyhydroxyalkanoate (PHA) oleh *Ralstonia eutropha* (dahulu dikenali sebagai *Alcaligenes eutrophus*). PHA dihasilkan melalui proses yang melibatkan dua tahap iaitu penghasilan asid laktik dari sisa buangan restoran diikuti dengan penghasilan PHA daripada asid laktik. Tahap pertama kajian difokuskan terhadap mengoptimumkan keadaan kultur untuk penghasilan asid laktik secara rawatan sesekelompok. Rawatan telah dijalankan menggunakan bioreaktor tangki berpengaduk bermuatan 2L dengan muatan bekerja sebanyak 1L. pH dikawal dengan menggunakan pengawal pH dengan penambahan 3M NaOH secara automatik, manakala asid laktik ditentukan dengan menggunakan teknik HPLC. Pengoptimuman keadaan yang paling baik bagi penghasilan asid laktik dilakukan terhadap suhu, tempoh masa pengawalan pH 7, kemasukan inokulum yang bersesuaian dan pengkulturan dibawah keadaan steril dan tidak steril. Dalam kebanyakan rawatan anaerobik, asid laktik dihasilkan secara dominan (70-99%) diikuti dengan asetik (1-20%), butirik (0-8%) dan asid propionik (0-1%). Diantara tiga

suhu berbeza yang dikaji, iaitu 30°C, 37°C dan 45°C, 37°C merupakan suhu yang terbaik untuk penghasilan asid laktik (17 g/L). Kajian ke atas kesan tempoh kawalan pH 7 menunjukkan bahawa dengan mengawal pH 7 selama 12 jam akan menghasilkan lebih banyak asid laktik (40 g/L). Penghasilan asid laktik berkurangan apabila pH 7 dikawal pada tempoh masa yang lebih panjang disebabkan oleh metanogenesis. Kemasukan *Lactobacillus rhamnosus* meningkatkan lagi penghasilan asid laktik (67 g/L). Peningkatan penghasilan asid laktik juga dikesan apabila rawatan dilakukan dalam keadaan steril. Sebagai rumusan, penghasilan asid laktik yang paling tinggi didapati daripada sisa buangan restoran adalah apabila suhu dikawal pada 37°C, kawalan pH 7 disepanjang tempoh eksperimen dan kemasukan *L. rhamnosus* serta dilakukan dalam keadaan steril.

Supernatan yang telah dikumpulkan daripada rawatan yang memberikan penghasilan asid laktik yang paling tinggi, digunakan dalam tahap kedua di mana polyhydroxyalkanoate dihasilkan. PHA dihasilkan oleh *Ralstonia eutropha* (*A. eutrophus*) strain ATCC 17699 secara kultur sesekelompok dengan menggunakan bioreaktor yang isipadu bekerjanya sebanyak 1L, menggunakan asid laktik sebagai sumber karbon semasa fasa penghasilan. Organisma yang digunakan dibiakkan terlebih dahulu di dalam medium yang kaya nutrien selama 24 jam sebelum dimasukkan ke dalam medium penghasilan yang mengandungi 20-25 g/L asid laktik sebagai sumber karbon. Ketumpatan sel inokulum pada peringkat awal ditentukan pada 4-5 g/L. Kepekatan maksimum PHA yang telah dihasilkan adalah lebih kurang 10 g/L dalam masa 70 jam, bersamaan 97% (g/g) berat kering sel.

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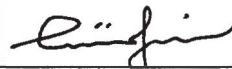
I certify that an Examination Committee met on 6th December 2001 to conduct the final examination of Hafizah Kassim on her Master of Science thesis entitled "Production of Lactic Acid and Polyhydroxyalkanoates (PHA) from Restaurant Waste" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

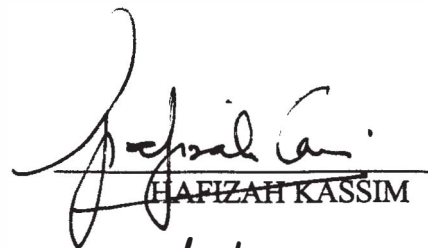

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CHAPTER I

INTRODUCTION

Environmental pollution has become a very important issue to the world for the past few decades, where the earth's environment has been deteriorating slowly but constantly by many factors including the disposal of non-biodegradable plastics, together with factors such as global warming and ozone layer depletion. Over the past decades, the intrinsic resistance of plastic materials to degradation has been increasingly regarded as a source of environmental and waste management problems (Lee and Yu, 1997). The world production of plastics is over one hundred million tons per year and these plastic materials account for about 20% by volume of municipal solid waste, therefore reducing the capacity of precious landfill sites (Chang, 1994). Ultimate treatments of plastic wastes are incineration and composting or landfill. But conventional plastic materials are not easily degraded in the environment because of their high molecular weight and hydrophobic characteristic. During the combustion of plastic wastes, hydrogen cyanide can be formed from acrylonitrile-based plastics and hydrogen chloride is released from polyvinylchloride (PVC) (Brandl *et al.*, 1990). Therefore, disposal of plastics has become a major environmental concern, resulting in programs to reuse or convert the waste (Kharas *et al.*, 1994).

In respond to the increasing public concern about the harmful effects of petrochemical-derived plastic materials in the environment, many countries are

conducting various solid-waste management programs, including plastic waste reduction by developing biodegradable plastic materials (Lee, 1996a). It has become of considerable industrial interest and of environmental importance to evaluate PHA as polyesters for the use in either biodegradable or biocompatible plastics for a wide range of possible applications (Brandl *et al.*, 1990). These biodegradable plastic materials must retain the desired material properties of conventional synthetic plastics, and should be completely degraded without leaving any undesirable residues when discarded (Lee, 1996a). The development and production of degradable plastics is rapidly expanding due to, in part, the growing legislation in developed countries aimed at barring the use of non-degradable plastics in a variety of consumer products (Leaversuch, 1987). But biodegradable plastics are still under development stage and thus not poses diverse physicochemical properties that common plastics have now, and secondly giant petrochemical industries are not much in a hurry to lose their current petrochemical markets to be replaced by biodegradable plastics.

Many types of biodegradable plastics which can be classified into partially or completely degradable plastics. PHAs or polyhydroxyalkanoates are example one of the completely degradable plastics which degrade to carbon dioxide and water within a few months of burial (Chang, 1994). Polyhydroxyalkanoates (PHAs) are polyesters of various hydroxyalkanoates which are synthesized by numerous microorganisms as an energy reserve material, usually when an essential nutrient such as nitrogen or phosphorus is limited in the presence of excess carbon source. PHAs are considered to be strong candidates for biodegradable polymer material because they are naturally

occurring and possess material properties similar to various synthetic thermoplastics and elastomers currently use (from polypropylene to synthetic rubber) and upon disposal, they are completely degraded to water and carbon dioxide (and methane under anaerobic conditions) by microorganisms in various environments such as soil, sea and lake water and sewage (Lee, 1996b; Byrom, 1994). Polyhydroxybutyrate (PHB) has received attention as a candidate for a novel biodegradable plastic material, since it has similar physico-chemical properties to polypropylene and polyethylene (Kim *et al.*, 1996). Currently PHA in the form of poly(3-HB-co-HV) or PHB/V is being produced on a large scale by Monsanto Ltd. In USA under the trade name 'Biopol' using *Alcaligenes eutrophus* H16. It cost US\$4.40/kg as compared to US\$0.60/kg for petrochemical plastic (Hassan *et al.*, 1997a).

One of the major problems that prevent the commercial application of PHAs is their high price. Much effort has been devoted to reduce the price of PHAs by the development of better bacterial strains, more efficient fermentation and more economical recovery process. Excluding the recovery process, the economics of PHAs production are largely determined by substrate cost and PHA yield. Among the various nutrients in the fermentation medium, the carbon source contributes most significantly to the overall substrate cost in PHA production. For practical application, cheaper carbon source needs to be utilized to reduce the production cost. The substrates usually used for the production of PHB are glucose, sucrose and fatty acids (Kim *et al.*, 1996). PHB can be produced from relatively cheaper substrates such as methanol, carbon dioxide, beet molasses or ethanol. Organic waste material such as restaurant waste, which is high in

organic carbon can also be a cheap carbon source for the production of value-added products. So far, very little research work have been done to this type of waste except as animal feed, composting or disposed off to landfills.

In this study restaurant waste was used as raw material for PHA production. The production of PHA from restaurant waste involved a two step process. The first step involved the controlled anaerobic treatment of restaurant waste. In this process, complex organic compounds are broken down by bacteria into simple organic acids especially lactic acid. The second step made use of the ability of a particular species of bacteria, *Ralstonia eutropha*, to consume the simple acids and convert them to intracellular biopolymers as energy and carbon reserve.

The objectives of this study were :

1. To optimise the culture conditions for enhancement the production of lactic acid from restaurant waste using anaerobic treatment by lactic acid bacteria.
2. To study the feasibility of using lactic acid obtained from the fermented restaurant waste for production of polyhydroxyalkanoates (PHA) by *Ralstonia eutropha*.

CHAPTER II

LITERATURE REVIEW

Municipal Solid Waste (MSW)

Introduction

The economic and demographic growth of Malaysia is posing serious challenges to the government especially to the urban authorities. It is increasingly stressing the environment, as the 'natural support system' that sustains the city, or as the effect of urbanization on the working and living conditions of others, such as downstream fishing communities. As the city experience rapid urban growth, environment degradation occurs in a large and growing problems of waste disposal, and more instance competition for increasingly congested spaces. The worsening urban environment is effecting people and nature in a number of ways including health, safety, productivity, amenity, and ecological integrity.

Developing countries such as Malaysia, do not have access to adequate sanitation. A significant amount of solid waste generated every day are either burned or end up in rivers, creeks, marshy areas and empty lots. Waste that is collected is mainly disposed off in open dump-sites, many which are not properly operated and maintained, thereby posing a serious threat to public health. Facilities for solid waste disposal is one such

infrastructure, which needs to be adequately provided to ensure the urban environment conducive to the well-being and productivity of residents. However, the management of this waste becomes a problem when the waste is concentrated or focused in a particular area with higher concentrations on the population economic activities.

In 1995, a total of 8,743 tonnes per day of urban waste generation in Malaysia, based on report prepared by Einsiedel (2000) on Final disposal of municipal solid waste, in Critical considerations of solid waste disposal in Asian cities. The capita generation rate was 0.81 kg per person per day. The increase in the income has resulted in a proportionate increase in consumption and consequently waste generation. In recent study of the World Bank, urban waste generation is predicted to increase substantially over the next coming years as GNP per capita increases. It is predicted that a total of 31.6 million tonnes per day waste generated in the next coming years (Einsiedel, 2000).

Definition

Municipal solid waste (MSW) includes wastes such as durable goods, nondurable goods, containers and packaging, food scraps, yard trimmings, and miscellaneous inorganic waste from residential, commercial, institutional, and industrial sources. Examples of waste from these categories include appliances, automobile tyres, newspapers, clothing, boxes, disposable tableware, office and classroom paper, wood pallets, and cafeteria waste. MSW does not include waste from other sources, such as

construction and demolition debris, automobile bodies, municipal sludge, combustion ash, and industrial process waste that might also be disposed in municipal waste landfills or incinerator (Village, 1998).

Food Waste

Food waste is considered as MSW. Food waste consists of uneaten food and food preparation wastes from residences, commercial establishments (restaurants, fast food establishments), institutional sources such as school cafeterias, and industrial sources such as factory lunchrooms. Food waste generated during the preparation and packaging of food products is considered as industrial waste and is not included in MSW food waste (Village, 1998). Food residuals comprise about 22 million tons of the MSW stream in United States and commercial generators add millions more to that volume. Not much have been done to this type of waste, but over 90% is disposed off to landfills. Traditionally food waste has been a source of livestock feed, but recently major plans are underway to develop composting programs and also a renewed interest in using these feedstock for animal feed (Lehto, 1999).

Kitchen leftover is characterized by a high organic content, and represents one waste stream for such exploitation. It contains cellulose, lipids, proteins and other compounds that are readily biodegradable and generally no compounds are found that are inhibitory to bacteria (Rintala and Ahring, 1994). In recent years, there has been a

growing interest in the fermentative treatment of the organic fraction of household refuse. According to Negri *et al.* (1993), a possible treatment of the organic fraction of municipal solid waste is the production of volatile fatty acids (VFA) through fermentation. Loh *et al.* (1999) produces organic acids from kitchen waste through anaerobic fermentation.

Management of MSW

Since in the 1960s and early 1970s, a large percentage of MSW was burned until mid-1980s. The burden on the landfills grew dramatically. In the developed countries the trend is to divert the large part of refuse away from landfill. To reduce the problem created by MSW, integrated waste management was planned which include:

1. Source reduction (including reuse of products and backyard composting of yard trimmings).
2. Recycling (including composting).
3. Waste combustion (preferably with energy recovery) and landfilled.

The predominant option for solid waste disposal is land disposal or landfill (Table 2.1).